

What is Claimed is:

1           1. A genetically modified astrocyte for gene  
2 therapy, said genetically modified astrocyte  
3 comprising:

4 one or more DNA sequences selected from the  
5 group consisting of DNA encoding a selectable marker,  
6 DNA encoding a poison pill, and DNA encoding a  
7 molecule useful for gene therapy; and  
8 suitable regulatory elements for controlling  
9 expression of said one or more DNA sequences.

1           2. The genetically modified astrocyte of claim  
2 1 wherein said selectable marker comprises neomycin  
3 resistance.

1       3. The genetically modified astrocyte of claim  
2       1 wherein said selectable marker comprises  
3       methotrexate resistance.

1           4. The genetically modified astrocyte of claim  
2       1 wherein said poison pill comprises herpes virus  
3       thymidine kinase.

1       5. The genetically modified astrocyte of claim  
2       1 wherein expression of said DNA encoding said  
3       molecule useful for gene therapy results in the  
4       production of a protein.

1        6. The genetically modified astrocyte of claim  
2        1 wherein expression of said DNA encoding said  
3        molecule useful for gene therapy results in the  
4        production of anti-sense RNA.

1        7. The genetically modified astrocyte of claim  
2        1 wherein expression of said DNA encoding said  
3        molecule useful for gene therapy results in the  
4        production of a ribozyme.

1        8. The genetically modified astrocyte of claim  
2        5 wherein said protein comprises a growth factor.

1        9. The genetically modified astrocyte of claim  
2        8 wherein said growth factor comprises a cytokine.

1        10. The genetically modified astrocyte of claim  
2        5 wherein said protein comprises tyrosine  
3        hydroxylase.

1        11. The genetically modified astrocyte of claim  
2        1 wherein said suitable regulatory elements include a  
3        regulatable promoter.

1        12. The genetically modified astrocyte of claim  
2        11 wherein said regulatable promoter comprises an  
3        inducible promoter.

1        13. The genetically modified astrocyte of claim  
2        12 wherein said inducible promoter comprises a human  
3        preproenkephalin promoter.

1        14. The genetically modified astrocyte of claim  
2        11 wherein said regulatable promoter comprises a  
3        constitutive promoter.

1        15. The genetically modified astrocyte of claim  
2        1 wherein said suitable regulatory elements include  
3        an astrocyte-specific promoter.

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1        16. The genetically modified astrocyte of claim  
2        15 wherein said astrocyte-specific promoter comprises  
3        a promoter for glial fibrillary acidic protein.

1        17. An astrocyte cell line comprising the  
2        genetically modified astrocyte of claim 1.

1        18. A plasmid for transfection of astrocytes  
2        which plasmid comprises DNA encoding a molecule  
3        useful for gene therapy and suitable regulatory  
4        elements for controlling expression of said molecule  
5        useful for gene therapy.

1        19. A plasmid for transfection of astrocytes  
2        which plasmid comprises DNA encoding a selectable  
3        marker and suitable regulatory elements for  
4        controlling expression of said selectable marker.

1        20. The plasmid of claim 19 further comprising  
2        DNA encoding a poison pill and further suitable  
3        regulatory elements for controlling expression of  
4        said poison pill.

1        21. A plasmid for transfection of astrocytes  
2        which plasmid comprises DNA encoding a poison pill  
3        and suitable regulatory elements for controlling  
4        expression of said poison pill.

1        22. An astrocyte stably transfected with one or  
2        more plasmids, said one or more plasmids selected  
3        from the group consisting of:

4              a plasmid comprising DNA encoding a molecule  
5        useful for gene therapy and suitable regulatory  
6        elements for controlling expression of said molecule  
7        useful for gene therapy;

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8        a plasmid comprising DNA encoding a selectable  
9        marker and suitable regulatory elements for  
10      controlling expression of said selectable marker;  
11      a plasmid comprising DNA encoding a selectable  
12      marker and suitable regulatory elements for  
13      controlling expression of said selectable marker, and  
14      further comprising DNA encoding a poison pill and  
15      further suitable regulatory elements for controlling  
16      expression of said poison pill; and  
17      a plasmid comprising DNA encoding a poison pill  
18      and suitable regulatory elements for controlling  
19      expression of said poison pill.

1        23. A method of stably transfecting primary  
2        cells, said method comprising stably transfecting  
3        said primary cells using non-viral transfection  
4        methods.

1        24. The method of claim 23 wherein said non-  
2        viral transfection method comprises chemical  
3        transfection.

1           25. The method of claim 24 wherein said  
2 chemical transfection comprises stable calcium  
3 phosphate transfection.

1        26. The method of claim 23 wherein said non-  
2        viral transfections method comprises electroporation.

1        27. The method of claim 23 wherein said primary  
2        cells comprise astrocytes.

1. 28 A method for gene therapy in the central  
2. nervous system of a subject which method comprises:

3           genetically modifying primary cells to include  
4   DNA encoding a molecule useful for gene therapy in  
5   the central nervous system;

6           transplanting said genetically modified primary  
7   cells into the central nervous system of a subject;  
8   and

9           expressing said DNA encoding said molecule,  
10   thereby producing said molecule for gene therapy in  
11   the central nervous system of the subject.

1           29. The method of claim 28 wherein said primary  
2   cells comprise astrocytes.

1           30. The method of claim 29 wherein said  
2   astrocytes are genetically modified by a non-viral  
3   transfection method.

1           31. The method of claim 30 wherein said non-  
2   viral transfection method comprises chemical  
3   transfection.

1           32. The method of claim 31 wherein said  
2   chemical transfection comprises stable calcium  
3   phosphate transfection.

1           33. The method of claim 28 wherein said  
2   expression of said DNA is controlled by a regulatable  
3   promoter.

1           34. The method of claim 33 wherein said  
2   regulatable promoter is controlled pharmacologically.

1           35. The method of claim 34 wherein said  
2   pharmacologic control comprises utilizing  
3   dopaminergic pathways.

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1           36. The method of claim 33 wherein said  
2        regulatable promoter comprises an inducible promoter.

1           37. The method of claim 33 wherein said  
2        regulatable promoter comprises a constitutive  
3        promoter.

1           38. A method of maintaining and growing  
2        astrocytes in culture, said method comprising:  
3           growing first astrocytes with a liquid medium  
4        overlying said first astrocytes so as to condition  
5        said liquid medium;  
6           removing said conditioned liquid medium; and  
7           placing said removed conditioned liquid medium  
8        over second astrocytes, said removed conditioned  
9        liquid medium capable of maintaining and growing said  
10      second astrocytes in culture.

1           39. A method of selecting for astrocytes in a  
2        mixed cell population, said method comprising:  
3           stably transfecting a mixed cell population with  
4        an astrocyte-specific plasmid, said astrocyte-  
5        specific plasmid comprising DNA encoding a selectable  
6        marker and suitable regulatory elements for  
7        controlling expression of said selectable marker;  
8           growing said transfected mixed cell population  
9        under selective conditions, wherein said astrocyte-  
10      specific promoter functions only in transfected  
11      astrocytes present in said transfected mixed cell  
12      population, such that only transfected astrocytes  
13      present in said transfected mixed cell population can  
14      be selected under said selective conditions using  
15      said selectable marker under control of said  
16      astrocyte-specific promoter; and

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17            selecting said astrocytes from said mixed cell  
18            population.

1            40. The method of claim 39 wherein said  
2            astrocyte-specific promoter comprises a promoter for  
3            glial fibrillary acidic protein.

1            41. The method of claim 39 wherein said  
2            selective marker comprises neomycin resistance.

1            42. The method of claim 39 wherein said  
2            selective marker comprises methotrexate resistance.

1            43. The method of claim 41 wherein said  
2            selective conditions include exposing said  
3            transfected mixed cell population to a neomycin  
4            analogue.

1            44. The method of claim 43 wherein said  
2            neomycin analogue comprises G418.

1            45. The method of claim 42 wherein said  
2            selective conditions include exposing said  
3            transfected mixed cell population to methotrexate.

1            46. A method of expressing a biologically  
2            active molecule in an astrocyte of a subject which  
3            method comprises:  
4                obtaining a sample of an astrocyte;  
5                stably inserting DNA encoding a biologically  
6                active molecule into DNA of said astrocyte;  
7                transplanting said resulting astrocyte into a  
8                subject; and  
9                expressing said biologically active molecule in  
10               said astrocyte in said subject.

1       47. The method of claim 46 wherein said  
2       biologically active molecule is selected from the  
3       group consisting of a protein, antisense RNA, and a  
4       ribozyme.

1       48. The method of claim 46 wherein said sample  
2       of an astrocyte is obtained by removing astrocytes  
3       from said subject.

1       49. The method of claim 46 wherein said stable  
2       insertion comprises a non-viral transfection method.

1       50. The method of claim 46 wherein said  
2       expression of said biologically active molecule is  
3       under control of a regulatable promoter.

1       51. The method of claim 50 wherein said  
2       regulatable promoter comprises an inducible promoter.

1       52. The method of claim 50 wherein said  
2       regulatable promoter comprises a constitutive  
3       promoter.

1       53. A method of killing astrocytes in a  
2       subject, said method comprising:  
3           obtaining a sample of astrocytes;  
4           stably transfecting said astrocytes with a  
5       plasmid, said plasmid comprising DNA encoding a  
6       poison pill and suitable regulatory elements for  
7       controlling expression of said poison pill;  
8           transplanting said transfected astrocytes into a  
9       subject; and  
10          exposing said transplanted transfected  
11       astrocytes to a selective condition, wherein said  
12       suitable regulatory elements cause expression of said

13 DNA encoding said poison pill only in said  
14 transplanted transfected astrocytes present in said  
15 subject such that only said transplanted transfected  
16 astrocytes present in said subject are killed by said  
17 selective condition due to said expression of said  
18 DNA encoding said poison pill under control of said  
19 astrocyte-specific promoter.

1 54. The method of claim 53 wherein said poison  
2 pill comprises herpes virus thymidine kinase.

1 55. The method of claim 54 wherein said  
2 exposure to a selective condition comprises exposure  
3 to a drug selected from the group consisting of  
4 acyclovir and gancyclovir.

1 56. A method of preventing deterioration of  
2 phenotypically normal cells in a subject which  
3 comprises:

4 detecting a genotype indicative of an eventual  
5 phenotypic abnormality in said normal cells;  
6 treating said normal cell with the genetically  
7 modified astrocyte of claim 1 so as to prevent said  
8 phenotypic abnormality, said prevention being by  
9 expression of said DNA encoding said molecule useful  
10 for gene therapy by said genetically modified  
11 astrocyte.

1 57. The method of claim 56 wherein said  
2 phenotypic abnormality is indicative of Huntingtons  
3 disease.

1 58. An astrocyte maintained and grown by the  
2 method of claim 38.

1       59. An astrocyte selected by the method of  
2       claim 39.

1       60. A kit for gene therapy comprising the  
2       genetically modified astrocyte of claim 1.

1       61. A kit for gene therapy comprising the  
2       genetically modified astrocyte of claim 17.

1       62. A kit for gene therapy comprising one or  
2       more plasmids, said one or more plasmids selected  
3       from the group consisting of:

4            a plasmid comprising DNA encoding a molecule  
5       useful for gene therapy and suitable regulatory  
6       elements for controlling expression of said molecule  
7       useful for gene therapy;

8            a plasmid comprising DNA encoding a selectable  
9       marker and suitable regulatory elements for  
10      controlling expression of said selectable marker;

11        a plasmid comprising DNA encoding a selectable  
12       marker and suitable regulatory elements for  
13       controlling expression of said selectable marker, and  
14       further comprising DNA encoding a poison pill and  
15       further suitable regulatory elements for controlling  
16       expression of said poison pill; and

17        a plasmid comprising DNA encoding a poison pill  
18       and suitable regulatory elements for controlling  
19       expression of said poison pill.

1       63. The kit of claim 62 further comprising  
2       astrocytes to be transfected with said one or more  
3       plasmids.

1       64. A kit for gene therapy comprising:

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2 a plasmid vector having a polylinker site for  
3 insertion of DNA encoding a gene of interest;  
4 restriction enzymes for inserting said DNA at  
5 said site; and  
6 the astrocyte of claim 58 to be transfected by  
7 the plasmid vector after insertion of said DNA into  
8 said plasmid vector.

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